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Title

Up-scaling aquaculture wastewater treatment by microalgal bacterial flocs: from lab reactors to an outdoor raceway pond

Running title

Up-scaling of MaB-floc reactors

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Abstract

Sequencing batch reactors with microalgal bacterial flocs (MaB-floc SBRs) are a novel approach for photosynthetic aerated wastewater treatment based on bioflocculation. To assess their technical potential for aquaculture wastewater treatment in Northwest Europe, MaB-floc SBRs were up-scaled from indoor photobioreactors of 4 L over 40 L and 400 L to a 12 m^3 outdoor raceway pond. Scale-up decreased the nutrient removal efficiencies with a factor 1-3 and the volumetric biomass productivities with a factor 10-13. Effluents met current discharge norms, except for nitrite and nitrate. Flue gas sparging was needed to decrease the effluent pH. Outdoor MaB-flocs showed enhanced settling properties and an increased ash and chlorophyll *a* content. Bioflocculation enabled successful harvesting by gravity settling and dewatering by filtering at 150-250 µm. Optimisation of nitrogen removal and biomass valorisation are future challenges towards industrial implementation of MaB-floc SBRs for aquaculture wastewater treatment.

Keywords

Algae; Bioflocculation; Raceway pond; Aquaculture wastewater; Biomass

1. Introduction

In recent years, intensive aquaculture has developed all over the world, bringing with it a whole new set of environmental concerns primarily concentrated around the issue of waste disposal (Martins et al., 2010; Richmond, 2004). Approximate 75 % of the aquaculture feed remains as nitrogen and phosphorous in the wastewater (Crab et al., 2007). During the past century, major efforts have been undertaken to remove these nutrients from aquaculture wastewater to obtain a satisfactory effluent quality that does not result in eutrophication of natural recipient ecosystems and/or that enables water recycling (Váradi et al., 2009; Crab et al., 2007). Nowadays, global warming and depletion of resources such as fossil energy, fresh water and phosphorous, mandates urgent efforts to redesign conventional wastewater treatment systems towards energy efficiency and nutrient recovery (Holmgren, 2002). Microalgae could play a key role in this redesign. Being photosynthetic microorganisms, they consume CO_2, lower the need for mechanical aeration by providing oxygen, scavenge resources from the wastewater (C, N and P) and convert solar energy into biomass (Van Den Hende et al., 2011a). A key factor determining the economic viability of microalgae systems for wastewater treatment is the cost-effective separation of microalgae from the treated water (Udom et al., 2013; Park et al., 2011a). In this regard, a novel approach based on bioflocculation of microalgae was developed: microalgal bacterial flocs in sequencing batch reactors (MaB-floc SBRs) (Van Den Hende et al., 2011a). MaB-flocs settle by gravity without the need for adding a flocculant. MaB-flocs settle in the reactor during night. Hereafter biomass-free effluent can be discharged. In this way, no expensive biomass removal techniques are needed for effluent discharge. Moreover, as the MaB-floc biomass is harvested, gravity settling provides a first concentration step of the MaB-floc biomass fraction.

On lab-scale, MaB-floc SBRs showed promising results for the treatment of pikeperch culture wastewater and concomitant biomass production (Van Den Hende et al., submitted a). Furthermore, MaB-floc biomass produced during these lab-scale experiments was efficiently harvested by a 200 µm pore filter press. On industrial scale, MaB-floc SBRs should be operated in outdoor raceway ponds, because raceway ponds tend to be the cheapest, the easiest to install and to operate, and the most durable microalgae culture systems (Richmond, 2004). In these shallow (0.4 m) algae raceway ponds, adequate nutrient removal rates are of importance because they determine the reactor volume and consequently the needed land area. Moreover, adequate MaB-floc settling and removal are needed to obtain a biomass-free effluent and to reach the target effluent norms. For biomass valorisation, the dominant presence of microalgae in MaB-flocs, the biomass productivity and its dewatering are of interest. Concerning up-scaling, lab-scale results should not be simply extrapolated to industrial outdoor scale. For example, extrapolation of data from treatment of pikeperch wastewater in a MaB-floc SBR of 4 L (Van Den Hende et al., submitted a) would result in an unrealistic biomass productivity of 160 ton Volatile Suspended Solids (VSS) ha^{-1} $year^{-1}$. Up-scaling to the outdoors brings along its specific outdoor operation conditions with respect to temperature (diurnal and seasonal fluctuations), light (intensity, spectral quality, photoperiod), reactor dimensions (depth, irradiated surface: reactor volume ratio, buffering), turbulence, contamination risk (dirt, algae grazers), harvesting equipment and wastewater composition variability (Richmond, 2004). Moreover, outdoor operation might require pH control by flue gas injection to reach the target discharge norm (< 9.5) and to avoid inhibition of activity of aerobic heterotrophic bacteria (optimum 8.3) (Park and Craggs, 2010; Oswald, 1988).

To assess the technical feasibility of outdoor MaB-floc SBRs for treatment of pikeperch wastewater in Northwest Europe, MaB-floc SBRs were up-scaled from indoor lab-scale

reactors (4 L, 40 L, 400 L) to an outdoor raceway pond (12 m^3). The key factors governing the technical feasibility of MaB-floc SBRs are evaluated: (1) wastewater treatment (nutrient removal, effluent quality, need for flue gas sparging), (2) MaB-floc characteristics (floc settling, chlorophyll and ash content), (3) biomass productivity, and (4) MaB-floc harvesting by gravity settling followed by filtering at 150-250 µm. Moreover, by comparing the reactor performances, a first assessment of the scale-up conversion factors to translate lab-scale results to outdoor scale in a raceway pond is provided.

2. Materials and methods

2.1 Indoor SBR of 40 L

The SBR with a working volume of 40 L was operated with a hydraulic retention time (HRT) of 2 days (Fig. 1.a), referred to as 40L_T2 (Table A.1, supplementary data). This reactor consisted of an open tank which was illuminated by one halogen lamp of 500 W (Silon CE-82-Y, Hong-Kong) and 3 halogen lamps of 20 W (Lunaqua3, Oase, Germany). The reactor was heated to 25 °C (HT 75W, TETRA, Germany). Peristaltic pumps were used for influent feeding and effluent withdrawal (both Watson Marlow, USA). The reactor was equipped with an overhead stirrer (100 rpm during days 1-2, 130 rpm during days 2-12, and 210 rpm during days 13-97) (Heidolph RZR 2020, Germany). No mechanical aeration of the reactor was performed. The aquaculture wastewater was drum filter effluent (30 µm) from indoor pikeperch culture (Inagro, Belgium), as earlier described (Van Den Hende et al., submitted a). This wastewater was sieved at 3 mm and stored at 4 °C until use. MaB-flocs from a previous experiment in a 4 L SBR treating pikeperch culture wastewater (Van Den Hende et al., submitted a) were up-scaled to 3 SBRs of 5 L during 2 months (no data shown) and used as inoculum for 40L_T2. From day 9 till 68, influent and effluent were sampled weekly whereas MaB-flocs were sampled 1-2 times a week. During days 69-97, 40L_T2 was operated to

produce MaB-floc inoculum for up-scaling. MaB-flocs were harvested 1-2 times a week to maintain 1 g Total Suspended Solids (TSS) L^{-1} and were stored at 4 °C to use as inoculum.

2.2. Indoor SBR of 400 L

The SBR with a working volume of 400 L was operated with a HRT of 4 days (Fig. 1.b.), referred to as 400L_T4 (Table A.1). The reactor was a plastic open tank illuminated by one halogen lamp of 500 W (Silon CE-82-Y, Hong-Kong) and 10 halogen lamps of 20 W (Lunaqua 3, Oase, Germany). The reactor was operated indoors (14-23 °C; Inagro, Roeselare) without heating. A centrifugal pump was used for influent feeding (Eco 3000 Aquarius Universal 30 W, Oase, Germany) controlled by a level regulator (REKA 2000, MAC3 Water Systems, Italy). A peristaltic pump (520SN/R2, Watson Marlow, USA) was used for daily effluent withdrawal after the MaB-floc settling phase at night. An overhead stirrer was used for mixing (53 rpm; T200020-0710, BCI Electromotoren nv, Belgium). No mechanical aeration of the reactor was performed. Pikeperch culture wastewater (same origin as for 40L_T2) was continuously fed to an influent tank after sieving at 4 mm and subsequent settling (Fig. 1b). Fish feed particles and faeces were removed 2-3 times a week from the sieve and settling compartments. The influent buffer tank was mixed every 0.25 h h^{-1} with a pump (Baseline, Maxeda DIY bvba, The Netherlands) to avoid sludge accumulation. The 400L_T2 reactor was inoculated with MaB-flocs at the start (25.80 g TSS, 19.75 g VSS), at day 7 (18.80 g TSS, 14.85 g VSS) and at day 18 (21.68 g TSS, 15.74 g VSS). MaB-flocs were frequently harvested to maintain 0.75 g TSS L^{-1} during period 1 (day 19-45, referred to as 400L_T4_0.75) and 0.50 g TSS L^{-1} during period 2 (day 46-98, referred to as 400L_T4_0.50) (Table A.1). Harvested MaB-flocs were stored at 4 °C until further use as inoculum. Twice a week, MaB-flocs, influent and effluent were sampled.

2.3. Outdoor SBR of 12 m^3

The SBR with a working volume of 11.959 m^3 was a raceway pond, referred to as 12M (Fig. 1c). This raceway was operated outdoors (50° 54’ 212’’ N, 3° 7’571’’ E; Inagro, Roeselare, Belgium). To enhance start-up during winter, the reactor was operated at a minimum temperature of 12 °C by a heating system consisting of warm water tubes and a gas boiler (Bulex, Belgium). Two propeller pumps (DRENO, Italy) stirred the raceway. Effluent was withdrawn with a submerged pump (Industrial pump system bvba, Belgium). The outdoor influent buffer tank was discontinuously stirred with a propeller pump (Dreno, Monselice, Italy; 6-7 periods of 0.08-0.75 h day^{-1} including during reactor feeding) to avoid sludge accumulation while wasting excess influent. Wastewater from pikeperch culture (same origin as 40L_T2) was mechanically pre-treated in an indoor settling tank. The first compartment of this settling tank removed particles larger than 1.2 mm by a vertical sieving screen. The second compartment removed settling and floating sludge. From here the water flowed to an indoor buffer tank and was pumped (emerged pump EUS, EVAK Taichung, Taiwan) to an outdoor influent buffer tank (HRT of all tanks was max. 3 days). Biweekly, excess fish feed and sludge were removed from the indoor tanks.

The reactor was operated for 231 days from winter to summer with 8 different operation periods (details are presented in Table A.1). Names of reactor operation periods are composed as ‘reactor volume in m^{3}’M_’operation period’_T‘HRT in days’_F’flue gas flow rate in L min^{-1}’. During start-up, the HRT of 8 days in period 1 (12M_1_T8_F0) was decreased to 4 days in period 2 (12M_2_T4_F0). At the start of period 3, the MaB-floc density in the reactor reached 0.5 g TSS L^{-1} and harvesting was started to maintain 0.5 g TSS L^{-1} (12M_3_T4_F0). Flue gas was sparged at 3 L min^{-1} during period 4 (12M_4_T4_F3) and 5 (12M_5_T8_F3) containing 214 ± 4 g CO_2 Nm^{-3}, 383 ± 8 mg NO Nm^{-3}, 572 ± 11 mg Nm^{-3} SO_2 (Lindegas, Belgium). Due to a temporary lack of wastewater volume, the HRT was increased to 8 days

during period 5 (12M_5_T8_F3) and 6 (12M_6_T8_F0). To demonstrate the need for flue gas sparging, no flue gas was sparged during period 7 (12M_7_T4_F0) and flue gas containing 89 ± 2 g CO_2 Nm^{-3} (Lindegas, Belgium) was sparged during period 8 (12M_8_T4_F5). During start-up, 12M was inoculated with 217, 38, 18, 46, 22, 134, 49 g TSS and 148, 32, 14, 33, 16, 102, 39 g VSS of MaB-flocs at day 1, 5, 8, 15, 18, 26, 35 and 44 respectively; being in total 0.044 g TSS $L_{reactor}^{-1}$ and 0.032 g VSS $L_{reactor}^{-1}$. The reactor DO (Visiferm DO probe, Hamilton, Belgium), reactor pH and reactor temperature ($T_{reactor}$) (201020/51-18-04 pH Pt 100 probe, Jumo, Germany), photosynthetic photon flux density (PPFD) (400-700 nm, PAR light sensor, LI-190, Li-Cor, USA), ambient temperature ($T_{ambient}$) (E+E Elektronik, Austria), reactor level (ultrasonic sensor, PEPPERL+FUCHS133990, Belgium), status of pumps, and flue gas flow rate (FGFR) (Mass-view CO_2 sensor, Mass-flow, Belgium) were logged minimum every 2 minutes. MaB-flocs, influent and effluent were sampled 2-3 times a week.

2.4. Harvesting of MaB-flocs

Harvesting of MaB-flocs from 12 M consisted of 2 steps: (1) concentration by settling and (2) dewatering by natural filtering followed by press filtering. In the first step, MaB-floc liquor was pumped with a flexible impeller pump (EP Midex 1400 L h^{-1}, Liverani, Italy) from the raceway pond into a 1 m^3 settling tank. After 1 h settling, supernatant (0.8 m^3) was pumped back in the raceway pond. In the second step, the remaining supernatant and settled MaB-flocs (0.2 m^3) were pumped in a linen filter bag (150-250 µm pore size; Lampe, Belgium). During pumping, this MaB-floc containing filter bag was first dewatered by gravity, resulting in a MaB-floc slurry and gravity filtrate. Thereafter, the slurry containing filter bag was further dewatered by hydropress (4 bar; Enotecnica Pillan, Italy) obtaining a MaB-floc cake and press filtrate. MaB-floc reactor liquor of the settling tank before settling, supernatant of the settling tank after settling, gravity filtrate and press filtrate were analysed for VSS and

TSS. MaB-floc losses during harvesting were calculated as the percentage of the total initial biomass in the buffer tank lost by settling, gravity filtering or press filtering. Dewatered MaB-flocs (cakes) were analysed for Total Solids (TS) and Volatile Solids (VS).

2.5. Analytical protocols

Water samples were analysed for pH, turbidity, Total Organic Carbon (TOC), Total Inorganic Carbon (TIC), Total Carbon (TC), Total Nitrogen (TN), Total Phosphorous (TP), Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD_5), and MaB-flocs samples were analysed for TSS, VSS, TS, VS, chlorophyll*a* (Chl*a*), physiological condition ($A664_b/A665_a$), autotrophic index (AI), diluted (dSVI), and by light and fluorescence microscopy, according to Van Den Hende et al. (submitted a). Electron conductivity (EC) was measured on water samples (K610, Consort bvba, Belgium). Filtered (0.2 µm RC 20/25, Chromafil, Germany) influent and effluent of 400L_T4_0.50 and 12M were analysed for NH_4^+, NO_2^-, NO_3^- and PO_4^{3-} with spectrophotometric test kits (Hach Lange, Belgium). Removal efficiencies and rates were calculated from daily influent and effluent values. The scale-up conversion factor for a certain parameter and reactor was calculated as the ratio of the average value of this parameter for this reactor divided by the average value of this parameter for the 12M reactor.

2.6. Statistics

Statistical analyses were performed using PASW Statistics 17.0 software (SPSS Inc, USA). Normal distribution of data was screened with a Shapiro-Wilk test and homogeneity of variances with a Levene's test. In case of normal data distribution and homogeneity of variances, significant differences were analysed by a ONE-WAY ANOVA and a Tukey's post-hoc test ($p < 0.05$). Otherwise, Kruskal-Wallis followed by a Mann-Whitney post hoc

test was used ($p < 0.05$). Averages are given with standard deviations. Correlations were quantified with non-parametric Spearman's r_s (two-tailed significance; $p < 0.005$).

3. Results and discussion

3.1. Wastewater treatment

3.1.1. MaB-floc SBR of 40 L

In reactor 40L_T2, the applied PPFD of 17 mmol PAR photons $L_{reactor}^{-1}$ day^{-1} was only 47 % of the average PPFD outdoors. Nevertheless, a significant decrease in turbidity, COD, BOD_5 and TOC in the MaB-floc SBR was observed (Fig. 2a). This resulted in a high removal rate (RR) and removal efficiency (RE) of these parameters (Table 1, 2). In contrast, the REs for TIC and EC were negative and showed strong variations (Fig. 3a; Table 2). The pH significantly increased from 7.31 ± 0.18 to 7.81 ± 0.11 (Fig. 3a). The obtained REs were sufficient to reach the current discharge norms for this wastewater for pH (6.0-9.5), COD (125 mg L^{-1}), BOD_5 (25 mg L^{-1}), TN (99.8 mg L^{-1}; sum of Total Kjeldahl N, N-NO_3^- and N-NO_2^-) and TP (24 mg L^{-1}) (Fig. 2a, 3a, 4a). Per g VSS produced, 1.99 ± 0.62 g COD, 1.08 ± 0.24 g BOD_5, 0.52 ± 0.17 g TOC, 0.35 ± 0.22 g TC and 0.22 ± 0.07 g TN were removed from the wastewater, and 16 ± 0.17 g TIC was net produced. This means that for each mol TN removed, 1.52 ± 1.43 mol TC and 2.38 ± 1.33 mol TOC was removed. The latter values are low compared to the TC: TN ratio of micro-organisms of approximately 6 (Geider and La Roche, 2002). This suggests that the N removal could not be due to biomass growth only. More research is needed to confirm whether denitrification in anoxic microniches of the flocs, as in activated sludge (Schramm et al., 1999), was an additional mechanism for N removal.

3.1.2. MaB-floc SBR of 400 L

To provide MaB-floc inoculum for the 12 m^3 reactor, harvesting was increased in the 400 L reactor, and the MaB-floc density was decreased from 0.75 g TSS L^{-1} in period 1 (400L_T4_0.75) to 0.50 g TSS L^{-1} in period 2 (400L_T4_0.50). During period 2, the influent turbidity was increased with a factor 3 and the influent COD, BOD_5, TOC, TN and TP concentrations were doubled compared with period 1 (Fig. 2b, 4b). The PPFD of 11 mmol PAR photons $L_{reactor}^{-1}$ day^{-1} was only 31 % of the average PPFD outdoors (Fig. 5). Nevertheless, during both operation periods, adequate RRs and REs were obtained (Table 1, 2). The effluent reached the current discharge norms for pH, COD, BOD_5, TN (except for 1 sample) and TP (Fig. 2b, 3b, 4b). In spite of the large variations of the influent turbidity, the effluent turbidity was rather stable. This might be due to the sequenced settling and/or dominance of a *Phormidium* sp. in the MaB-flocs. These filamentous cyanobacteria can release turbidity removing bio-flocculants (Bar-Or and Shilo, 1987), and acted like a filtering net during MaB-floc settling. The TIC was only significantly removed during period 2 (Fig. 3b). During both operation periods, no significant decrease in EC and increase in pH was observed (Fig. 3b). Therefore, flue gas sparging was not needed for pH control.

The molar ratios of removal rates are in line with the average TC: TN ratio and TP: TN ratio of microbial biomass (Geider and La Roche, 2002). Indeed, for each mol TN removed, 10.8 ± 11.4 mol TC was removed (averages of periods 1 and 2), and for each mol TP removed, 9.6 ± 8.4 mol TN was removed. However, per g VSS produced, 0.49 ± 0.42 g TN, 6.62 ± 3.80 g COD, 3.59 ± 2.90 g BOD_5, 1.08 ± 1.43 g TOC and 2.08 ± 2.56 g TC, 1.00 ± 1.64 g TIC and 0.15 ± 0.08 TP were removed (averages of period 1 and 2). This means that not all C and N removal could have been due to biomass growth. More research is needed to confirm which of the following removal mechanisms play a role in this: TN removal via denitrification, VSS removal by predators, TC removal via CO_2 emission to air and $CaCO_3$ precipitation.

During period 2, nitrogen species were studied more in detail (Fig. 4b). On average, 97.8 ± 3.0 % of NH_4^+-N and 81.3 ± 23.0 % of NO_2^--N were removed. This resulted in effluent containing 0.15 ± 0.18 mg NH_4^+-N L^{-1} and 0.17 ± 0.18 mg NO_2^--N L^{-1}, and largely met the current discharge norms of 32 mg NH_4^+-N L^{-1} and 0.91 mg NO_2^--N L^{-1}. In contrast, the nitrate concentration of the effluent (55.0 ± 8.60 mg N-NO_3^- L^{-1}) was largely above the current discharge norm of 33.9 mg N-NO_3^- L^{-1}. The N-NO_3^- concentration in the wastewater was on average with 18.2 ± 15.7 mg N-NO_3^- L^{-1} increased during treatment in the MaB-floc SBR (Fig. 4b). The question remains whether this was due to nitrification, excretions by MaB-floc predators and/or lysis of organic compounds catalyzed by enzymes excreted by cyanobacteria, such as protease and urease (Thajuddin and Subramanian, 2005).

3.1.3. Outdoor MaB-floc SBR raceway pond of 12 m^3

Large diurnal fluctuations in the pH and DO concentrations of the raceway pond (referred to as 12M) were observed (Fig. 5). These are typical for photosynthetic algae reactors (Richmond, 2004), but can lead to a reactor pH above the discharge norm of 9.5. Therefore, effluent was discharged after the pH decrease at night. During summer days with increased daily PPFD (Fig. 5), the pH decrease during dark was not sufficient to reach the pH discharge norm of 9.5 (Fig. 3c). Therefore flue gas sparging was needed. This was demonstrated by turning off the flue gas sparging during period 6 and 7, resulting in an effluent pH of 9.78 ± 0.46 and 9.78 ± 0.51 respectively (Fig. 3c). Sparging 5 % CO_2, representing flue gas of natural gas burning (Van Den Hende et al., 2012), at 0.00039 vvm during period 8 in 12M led to a dischargeable effluent pH of 9.08 ± 0.22. Compared to previous studies with flue gas sparged MaB-floc reactors (Van Den Hende et al., 2011a), low volumetric flue gas loading rates (FGLR) were applied. This is of importance because a decreased FGLR means (1) less gas pumping and thus lower energy and maintenance costs, and (2) a smaller number of flue

gas blower systems in the raceway pond and thus decreased capital and maintenance costs. Moreover, by applying a low FGLR, an increase in TIC in the effluent was avoided (Fig. 3c). The RR of TIC doubled in time (Table 1). The main TIC removal mechanism was not the biomass productivity, because these parameters were not significantly correlated. The TIC RR only poorly correlated with the daily PPFD ($r_{sp} = 0.296$, $p < 0.05$). Photo-saturation and photo-inhibition at high PPFDs (Richmond, 2004), varying TIC production in the reactor and/or TIC removal via $CaCO_3$ precipitation could explain this. Similar to the TIC RR, the EC RR increased in time (Table 1). The TIC RR was positively correlated with the EC RR ($r_s = 0.454$, $p < 0.005$), the ash content of MaB-flocs ($r_s = 0.564$, $p < 0.001$) and the $T_{reactor}$ ($r_s = 0.624$, $p < 0.001$). These observations support our hypothesis that the increased TIC removal was mainly due to carbon scavenging by $CaCO_3$ precipitation which increases by increasing temperature and which lowers the EC by removing calcium.

The COD and BOD_5 of the effluent reached the current discharge norms, but their influent concentrations were in most cases already below the discharge norms (Fig. 2c). The influent COD (56 ± 35 mg L^{-1}) showed strong variations in time (Fig. 2c). This led to low and strongly varying REs (Table 1). The loading rates of 12 ± 7 mg COD $L_{reactor}$ day^{-1} and 2 ± 2 mg BOD_5 $L_{reactor}$ day^{-1} were very low. This resulted in DO concentrations in reactor 12M above 5 mg L^{-1} during most of the operation period, even at night, except during period 8 (Fig. 5). The supersaturated DO values up to 24 mg L^{-1} (Fig. 5) demonstrate photosynthetic aeration by MaB-flocs and BOD_5 underloading of 12M. These supersatured DO values can be toxic for microalgae (Richmond, 2004). Therefore, during further optimisation research should investigate the potential of increased BOD_5 loadings on the reactor performance, for example by altering the influent pre-treatment step.

The influent TN concentration of reactor 12M decreased in time from 68.4 ± 12.4 g L^{-1} in period 2 to 32.7 ± 11.7 g L^{-1} in period 8; all of which are above the discharge norm (Fig. 4c). On average, only around 50 % of TN was removed in reactor 12M (Table 2). The TN RR was not significantly correlated with the daily PPFD or any other nutrient removal rates. All influent and effluent concentrations of N-NH_4^+ were below the discharge norm (Fig. 4c). The NH_4^+ RE varied during period 2-3, but was more stable during period 4-8 (Fig. 4c). The NO_3^- in the influent varied in time and reached values above the discharge norm (Fig. 4c). The NO_3^- RE was negative during the start-up in period 2, but increased in time from period 3 to 8 (Fig. 4c). The current discharge norms for NO_3^- were met, except during period 2, 3 and 4. Since the NO_3^- RR was positively correlated with the PPFD ($r_s = 0.499$, $p < 0.001$), $T_{reactor}$ ($r_s = 0.429$, $p < 0.005$) and the RR PO_4^{3-} ($r_s = 0.429$, $p < 0.005$), the weather conditions and/or a phosphorous limitation could be a reason for the decreased NO_3^- RR.

The influent contained on average 2.50 ± 1.83 mg N-NO_2^- L^{-1} which is largely above the discharge norm (Fig. 4c). Only during period 3, the effluent met the discharge norm for N-NO_2^- (Fig. 4c). The NO_2^- RE decreased in time (Fig. 4c). Negative NO_2^- REs were obtained, despite the aerobic conditions needed for nitrification. Further optimization to lower the NO_2^- concentrations is crucial. Since the NO_2^- concentration in the indoor fish tanks was lower than 1 mg L^{-1}, the NO_2^- must have increased during storage in the influent buffer tanks. Therefore a possible strategy to enhance the NO_2^- of the effluent would be to decrease the HRT of the influent buffer tanks by discontinuous feeding of the MaB-floc SBR during the day.

Very low average effluent values of 0.80 ± 0.60 mg TP and 0.54 ± 0.60 mg P-PO_4^{3-} L^{-1} were obtained; both below their discharge norm (Fig. 4c). These values decreased in time, reaching a minimum during period 7 (0.42 ± 0.23 mg TP L^{-1} and 0.11 ± 0.10 mg P-PO_4^{3-} L^{-1}). The PO_4^{3-} RR was positive correlated with the daily PPFD ($r_s = 0.486$, $p < 0.001$) and $T_{reactor}$

($r_s = 0.462$, $p < 0.005$). For each mol TP removed, 26.1 ± 20.5 mol TN was removed. These results together with the high TN: TP ratio of the effluent (128 ± 60) suggest that P limited the N removal via biomass growth. The above results highlight the large potential of MaB-floc SBRs for P polishing and recovery, of large interest nowadays (Shilton et al., 2012).

3.1.4. Effect of up-scaling on wastewater treatment

In spite of the varying wastewater composition, biomass density and HRT, some general conclusions can be made on the effect of up-scaling of MaB-floc SBRs on wastewater treatment. Firstly, up-scaling to outdoor conditions strongly increased the effluent pH. Outdoors, flue gas sparging was needed to obtain an effluent pH below the discharge norm. Despite being advantageous in terms of greenhouse gas mitigation, flue gas sparging in wastewater treatment raceways represents an extra cost (up to 20 % of the capital expenditures; Zamalloa et al., 2011). In certain countries, such as Belgium, this cost cannot be compensated by revenues for CO_2 credits because no such credits can be obtained for flue gas scrubbing in open ponds.

Secondly, up-scaling not always decreased, but for some nutrients increased the nutrient removal (Table 1, 2). Indeed, the TIC RR in 12M was in the same order as in the 4 L and 400 L reactor, but was higher than the negative values obtained in the 40 L reactor. In winter, the EC RR of 12M was similar as these values of the 400 L reactor, but in summer these values increased with a factor 6. The same trend was observed for the TIC RE. Up-scaling to outdoor raceway pond decreased the RR of TOC, COD and BOD_5 with a factor up to 46. This was mainly due to their decreased concentrations in the influent during up-scaling. In contrast, the REs of these parameters were decreased with a factor 2-3 while up-scaling. The TN RR outdoors were decreased with a factor 3 compared to the 4 L and 40 L reactors, but was similar to the 400 L reactor. The TN RE in 12 M decreased only with a factor 1-2 compared

to the 4 and 40 L reactor. The TP followed the same trend. In general, the scale-up conversion factors were less varying for the RE compared to those for the RR. This means that extrapolation of wastewater treatment reactor performances from lab to outdoor pilot scale, as a first rough assessment, should be based on RE rather than on RR. Overall, the nutrient REs from the wastewater were decreased with a factor 1-3 during up-scaling. Third, up-scaling increased the HRT to 4 days. This means that per m^3 indoor fish tank, a microalgae raceway pond area of 1 m^2 would be needed (daily discharge of 10 % of the fish tank water). To overcome potential problems of regarding shortage of availability of land, raceway ponds on the roof of the indoor aquaculture facility could be a worthwhile option to investigate. If year round wastewater treatment is targeted, pond heating with waste heat will be needed.

3.2. MaB-floc characteristics and effect of up-scaling

A first important requirement of MaB-flocs is adequate settling, because it is crucial for safeguarding the discharge of biomass-free effluent and high biomass recovery. This means that at the end of the dark period of the SBR operation, the volume of the settled MaB-flocs should be lower than the effective reactor volume after effluent withdrawal. In all MaB-floc SBRs, this was obtained. Indeed, the fact that maximum 50 % of the effective reactor volume was withdrawn during effluent discharge, that the average dSVI of all SBRs was lower than 250 mL g^{-1} TSS (Fig. 6a) and that the TSS values were lower than 2 g TSS L^{-1} (Fig. 6e, 6f) confirms this. The dSVI significantly decreased while up-scaling from 4 over 40 to 400 L, but significantly increased again in 400L_T4_0.50 (Fig. 6a). This was related to the dominance of filamentous cyanobacteria. In 12M, the dSVI (Fig. 6a) and settled MaB-floc density (Fig. 6b) remarkably decreased in time. This dSVI showed a strong positive correlation with the VSS/TSS ratio of the MaB-flocs ($r_s = 0.935$; $p < 0.001$) (Fig. 6g). The VSS/TSS ratio decreased in time from 79.1 ± 1.5 % in 12M_02_T2_F0 to 30.7 ± 3.1 % in 12M_08_T2_F5

(Fig. 6c). Microscopic observations showed that a large amount of crystals were present in the MaB-flocs (Fig. A.1). Further research is needed to confirm the nature of these crystals.

For biomass dewatering and valorization, the size, structure and abundance of the most dominant photosynthetic micro-organisms (PM) present in MaB-flocs is of importance. During up-scaling, the dominant PM in MaB-flocs changed from filamentous cyanobacteria (*Phormidium* sp.) indoors to filamentous microalgae (*Ulothrix* sp. or *Klebsormidium sp.*) in 12M (microscopic observations; Fig. A.1). Filamentous PM are suitable candidates for wastewater treatment, because they can be harvested relatively easily (Markou and Georgakis, 2011). To the best of our knowledge, this is the first report on dominance of this microalgae species in an outdoor raceway. Although *Ulothrix* sp. are not commercially cultured, these species can be interesting in aquaculture to increase fish fertility and as source of unsaturated fatty acids 16:4 ω-3 and 18:3 ω-3 (Jameison and Reid, 1976). Moreover, *Ulothrix* sp. contain a high amount of antibiotics, chlorophyll and carotenoids (Jaya Prakash Goud *et al.,* 2007). During spring, the growth of *Scenedesmus dimorphus* in the outdoor influent tank led to the unwanted presence of this non-flocculating species in the effluent. Covering the influent tank, solved this problem. This demonstrates that SBR operation can avoid the dominance of non-flocculating microalgae in a MaB-floc raceway pond, even with a high HRT of 8 days. Up-scaling to 400 L significantly decreased the Chl*a* content of the MaB-flocs (Fig. 6d). This was probably due a combination of the decreased PPFD (Table A.1) and increased BOD_5 loading (Fig. 2a, b), favoring the growth of bacteria. In contrast, in 12M, the Chl*a* content of MaB-flocs increased from 0.66 ± 0.13 % of VSS in 12M_02_T4_F0 to 1.64 ± 0.54 % of VSS in 12M_08_T4_F5 (Fig. 6d). These values are in the upper range of those of pure microalgae or cyanobacteria (0.17 to 4.36 % of TS; Piorreck *et al.*, 1984) and higher than that of earlier reports for *Ulothrix* sp. (0.56 % of TS; Jaya Prakash Goud *et al.,* 2007) and *Phormidium* sp.

(0.01-0.16 % of TS) (Bhattacharya and Pal, 2012). This indicates a high microalgae content in MaB-flocs, a hypothesis which was confirmed by microscopy (Fig. A.1). The average A664b/A665a ratio was above 1.63 in all reactor set-ups. This demonstrates an adequate physiological condition of the PM in all SBRs (Van Den Hende et al, 2011a). It indicates that nor the increased ash content of MaB-flocs, nor sparging of flue gas containing NO and SO_2, which can be toxic to some algae species (Van Den Hende et al., 2012), were severely toxic for the MaB-flocs.

To conclude, up-scaling from indoor reactors to an outdoor raceway pond led to a drastic shift in the community structure, increased the ash and Chl*a* content of MaB-flocs and enhanced the floc settling. These changes highlight the importance of outdoor pilot scale studies especially with respect to floc settling and biomass valorization perspectives.

3.3. MaB-floc productivity

The average MaB-floc productivity was low (Table 3), resulting in 9.2 g TSS and 3.2 g VSS per m^{-2} pond area day^{-1}, or 33 ton TSS and 12 ton VSS ha^{-1} pond area year^{-1}. Compared with indoor experiments, these values are 10-13 times lower, except for 400L (Table 3). They are 1.5-4 times lower compared to the result of 13-35 g TS m^{-2} pond area day^{-1} obtained by Park et al. (2011b) for wastewater treating algae raceway ponds. One of the reasons for this was the temporarily increased HRT. Indeed, during periods 5 and 6 of 12M with both a HRT of 8 days, the biomass productivity was only 70 and 20 % of the average productivity in 12M, respectively. In summer (period 7 and 8), 16 g TSS and 5 g VSS m^{-2} day^{-1} were produced This is still lower than 25 g m-^{2} day^{-1} obtained during summer by Park and Cragss (2010) in a wastewater treating algae pond. Another reason was biomass loss due to predators. The fact that 25 % of the measured biomass productivities (68 in total) were negative and MaB-floc predators were observed in 12M (mainly larvae of *Tubifex* sp.; around 10-20 per kg dewatered

MaB-flocs), confirms this. The presence of *Tubifex* sp. in the biomass can be beneficial for valorization as aquaculture feed ingredient, since *Tubifex sp*. can increase appetites and palatability of fish (Lietz, 1988).

The MaB-floc productivities showed no significant positive correlation with the RRs of C, N and P parameters. This discrepancy could be due to biomass loss by predators, as observed in previous studies (Weinberger et al., 2012; Mulbry et al., 2008) and/or nutrient removal via other mechanisms such as denitrification, precipitation and adsorption (Shilton et al., 2012). To be realistic, until a sustainable and lucrative pathway of biomass valorization is demonstrated, an increased biomass production per m^3 wastewater is actually not wanted, since this would only represent extra costs for MaB-floc harvesting and dewatering.

The maximum quantum yields (Y_{qmax}) were calculated as daily mol C-VSS produced per daily mol PAR photons provided, assuming that the by microalgae dominated VSS contained 50 % C (Van Den Hende et al., 2012). This Y_{qmax} is not the actual but a maximum possible value, because VSS could also have been produced heterotrophically or added with influent feeding. Since in photosynthesis, 8 mol photons are needed to convert 1 mol inorganic C to organic C, and around 10-20 % of the light is lost due to reflection on the pond surface (Park et al., 2011b), maximum Y_{qmax} values of 8-9 % are expected in case of no photo-saturation like in indoor reactors. The high values obtained in 40L_T2 (Table 3) demonstrate that VSS productivity in this reactor was not only due to photosynthetic growth. Since photosynthesis of most algal species is saturated at a PPFD of around 200 µmol m^{-2} s^{-1}, which is about 10-17 % of summer/winter maximum outdoor PPFD (Park et al., 2011b), more realistic Y_{qmax} values for outdoor reactors range between 0.8 % and 1.5 %. The Y_{qmax} obtained for 12M (Table 3) was 1.1 ± 1.0 % and thus in this range. This theoretical outdoor Y_{qmax} value (Park et al., 2011b) and outdoor Y_{qmax} value calculated for 12M don’t not take into account pond shading

by pond walls or adjacent trees or buildings. In the presented study, pond shading by trees and buildings was neglectable, but pond shading by pond walls could have played a role (Velmurugan and Srithar, 2008), especially during winter. The 60 % lower Y_{qmax} values obtained during period 5 and 6 (Table 3) with a doubled HRT indicate that light was not the reason for the decreased biomass productivity during these periods. Overall, up-scaling lowered the Y_{qmax} values to realistic values for outdoor photosynthetic growth.

3.4. MaB-floc harvesting

An efficient and cost-effective biomass harvesting is a key factor for a sustainable wastewater treatment by microalgae (Udom et al. 2013; Park et al., 2011). In this study, harvesting of MaB-flocs proceeded in two steps: (1) concentration by gravity settling in a settling tank, and (2) dewatering of the settled biomass by filtering. For cost-effective harvesting of MaB-flocs on an industrial scale, it is important that floc settling is fast and that it leads to high enough MaB-floc densities, to reduce the settling tank dimensions and pumping. Both on lab and on pilot scale, MaB-flocs settled fast. On lab-scale, the settled MaB-floc densities ranged between 4 and 8 g TSS L^{-1} and 2 and 6 g VSS L^{-1} (Fig. 6b). In 12M, these values increased from 4.5 ±1.0 g TSS L^{-1} and 3.5 ± 0.8 g VSS L^{-1} in period 2 to the 70.3 ± 16.2 g TSS L^{-1} and 21.1 ± 3.3 g VSS L^{-1} in period 7 (Fig. 6b). Gravity settling during harvesting in reactor 12M increased the MaB-floc TSS densities with a factor up to 90. Settling was the harvesting step with the highest MaB-floc loss (Table 4). The MaB-floc losses were still in the same range to lower compared to microalgae settling by flocculant addition (2-15 % TSS, after 1 h settling) (Udom et al., 2013) and by bioflocculation in a biomass recycling raceway (14-75 % TSS, yearly average after 1 h settling) (Park et al., 2011a). In the presented study, the remaining biomass in the supernatant was actually not lost, because it was pumped back into the pond to

enable pond stirring without decreasing the HRT (i.e. adding extra influent) and to increase biomass recovery during the 10 times longer settling period at dark.

During the second harvesting step, the settled MaB-flocs were pumped in a filter bag and dewatered by gravity filtering followed by hydropress filtering. A relatively large pore size of 150-250 µm was used (Uduman et al., 2010). Dewatering increased the TSS densities of settled MaB-flocs with a factor up to 27. Dewatering led to a MaB-floc cake of 25-50 %TS (Table 4). This is higher compared to the resulting 21 %TS in a previous lab-scale study (Van Den Hende et al., submitted a), and in the mid-range compared to 3-90 %TS obtained for other algae dewatering systems (Uduman et al., 2010; Udom et al. 2013). During dewatering, the loss of VSS was always higher than of TSS (Table 4). As for gravity filtering, this was due to the loss of microalgae passing through the filter pores. As for press filtering, no microalgae were observed in the filtrate but the orange filtrate color suggests leakage of cell content due to the applied high pressure of 4 bar. On industrial scale, a lower pressure of the filter press will be applied. The gravity filtrate and press filtrate had a volume of 18 % and 1.5 % of total harvested reactor liquor. The filtrate contained 0.044 ± 0.025 and 0.046 ± 0.044 g TSS L^{-1}, and 0.029 ± 0.017 and 0.033 ±0.031 VSS g L^{-1}, respectively. This resulted in a discharged filtrate containing 0.044 ± 0.023 g TSS L^{-1} and 0.029 ± 0.016 g VSS L^{-1}. This is largely under the current discharge norm for this pikeperch culture wastewater of 1 g SS L^{-1}. In this study, a hydropress was tested which uses water pressure of 4 bar and no electricity. Per kg dry biomass, 40 L of water was needed to dewater 1 kg of biomass. This has a cost (if drinking water in Belgium) of around 4 € m^{-3} or 0.16 € kg^{-1} dry MaB-flocs. On industrial scale, an electricity-powered belt filter press will be a cheaper option, having an operational cost of 0.4-0.7 kWh kg^{-1} dry matter (Udom et al., 2013) and resulting in 0.04-0.07 € kg^{-1} dry matter. To conclude, despite the drastic change of dominant microalgae species in the MaB-

flocs, a floc recovery of 98.8 ± 0.9 % of TSS and 98.0 ± 1.5 % of VSS showed similar results compared to lab-scale. MaB-floc dewatering showed better results compared to lab-scale. These promising results warrant further up-scaling with industrial belt filter presses.

4. Conclusions

MaB-floc SBRs treating aquaculture wastewater were up-scaled from indoor reactors to a 12 m^3 outdoor raceway pond. This scale-up decreased the nutrient removal efficiency and biomass productivity with a factor 1-13. Current discharge norms were reached, except for nitrate and nitrite. Outdoors, flue gas sparging was needed to lower the effluent pH. Both settling and ash content of MaB-flocs strongly increased during summer. Bioflocculation enabled successful harvesting by gravity settling and dewatering by filtering at 150-250 µm. Future research should focus on nitrogen removal and biomass valorisation.

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Table 1. Removal rates of MaB-flocs SBRs treating aquaculture wastewater and scale-up conversion factors compared to 12M

Reactor	Removal rates (unit $L_{reactor}^{-1}$ day^{-1}) and scale-up conversion factors[c]						
	EC ($\mu S\ cm^{-1}$)	TIC (mg)	TOC (mg)	COD (mg)	BOD_5 (mg)	TN (mg)	TP (mg)
4L_T2[a]	n.d.[b]	15.0 ± 7.0 (1.5)	14.0 ± 4.1 (4.7)	65 ± 6 (16)	19 ± 2 (18)	9.8 ± 1.8 (2.7)	1.56 ± 0.08 (5.4)
40L_T2	-41 ± 38 (-1.7)[c]	-7.5 ± 6.5 (-0.8)	19.5 ± 8.5 (6.6)	88 ± 26 (22)	49 ± 11 (46)	10.3 ± 2.7 (2.8)	1.73 ± 0.73 (6.0)
400L_T4_0.75	3 ± 4 (0.1)	2.6 ± 9.5 (0.3)	12.6 ± 7.5 (4.2)	38 ± 29 (9.5)	44 ± 20 (42)	4.2 ± 4.2 (1.2)	0.67 ± 0.29 (2.3)
400L_T4_0.50	9 ± 9 (0.4)	20.4 ± 13.6 (2.1)	21.8 ± 16.8 (7.4)	79 ± 29 (20)	44 ± 21 (42)	6.2 ± 4.5 (1.7)	2.10 ± 1.08 (7.2)
12M_02_T4_F0[d]	5 ± 9 (0.2)	5.9 ± 5.4 (0.6)	5.6 ± 7.4 (1.9)	7 ± 4 (1.7)	3 ± 2 (2.9)	5.7 ± 4.0 (1.6)	0.18 ± 0.09 (0.6)
12M_03_T4_F0[d]	-2 ± 7 (-0.1)	7.6 ± 2.2 (0.8)	3.1 ± 3.0 (1.1)	4 ± 7 (0.9)	0 ± 4 (0)	3.4 ± 1.2 (0.9)	0.35 ± 0.10 (1.2)
12M_04_T4_F3[d]	15 ± 0 (0.6)	7.4 ± 3.1 (0.8)	3.5 ± 0.5 (1.2)	13 ± 13 (3.1)	1 ± 1 (0.6)	3.0 ± 3.1 (0.8)	0.31 ± 0.04 (1.1)
12M_05_T8_F3[d]	16 ± 2 (0.7)	5.3 ± 0.1 (0.5)	0.8 ± 0.1 (0.3)	9 ± 16 (2.4)	1 ± 1 (0.7)	1.4 ± 0.2 (0.4)	0.49 ± 0.28 (1.7)
12M_06_T8_F0[d]	25 ± 15 (1.0)	4.5 ± 1.0 (0.5)	2.3 ± 3.3 (0.8)	3 ± 4 (0.8)	0 ± 0 (0)	2.7 ± 1.8 (0.7)	0.25 ± 0.05 (0.9)
12M_07_T4_F0[d]	45 ± 21 (1.9)	15.2 ± 3.6 (1.6)	0.9 ± 7.4 (0.3)	-2 ± 4 (-0.5)	0 ± 1 (0)	3.5 ± 3.2 (1.0)	0.35 ± 0.10 (1.2)
12M_08_T4_F5[d]	41 ± 24 (1.7)	14.2 ± 2.7 (1.5)	3.1 ± 4.2 (1.0)	3 ± 5 (0.7)	1 ± 1 (0.8)	3.4 ± 2.5 (0.9)	0.31 ± 0.11 (1.1)
12M_all data[e]	24 ± 24 (1.0)	9.8 ± 5.8 (1.0)	3.0 ± 5.4 (1.0)	4 ± 7 (1.0)	1 ± 2 (1.0)	3.6 ± 3.0 (1.0)	0.29 ± 0.13 (1.0)

[a] Results of a MaB-floc sequencing batch reactor of 4 L with hydraulic retention time of 2 days (Van Den Hende et al., submitted a); [b] No data; [c] Values between brackets are scale-up conversion factors calculated as average removal rate for a certain reactor divided by the average removal rate of 12M_all; [d] Names of reactor operation periods are composed as 'reactor volume in m^3'M_'operation period'_T'HRT in days'_F'flue gas flow rate in L min^{-1}'; [e] Average of all samples of period 2-8 of reactor 12M

Table 2. Removal efficiencies of MaB-flocs SBRs treating aquaculture wastewater and scale-up conversion factors compared to 12M

Reactor	Removal efficiencies (%) and scale-up conversion factors[c]						
	EC	TIC	TOC	COD	BOD_5	TN	TP
4L_T2[a]	n.d.[b]	36 ± 17 (0.8)[c]	71 ± 21 (2.1)	80 ± 7 (2.9)	87 ± 11 (1.6)	58 ± 11 (1.8)	89 ± 5 (1.4)
40L_T2	-15 ±13 (-1.0)	-15 ± 16 (-0.3)	63 ± 22 (1.9)	80 ± 10 (2.8)	99 ± 2 (1.9)	41 ± 8 (1.3)	65 ± 15 (1.0)
400L_T4_0.75	2 ± 2 (0.1)	7 ± 31 (0.2)	73 ± 31 (2.2)	68 ± 21 (2.4)	95 ± 11 (1.8)	29 ± 25 (0.9)	59 ± 13 (0.9)
400L_T4_0.50	4 ± 4 (0.3)	45 ± 16 (1.0)	77 ± 20 (2.3)	93 ± 6 (3.3)	98 ± 4 (1.8)	22 ± 20 (0.7)	70 ± 12 (1.1)
12M_02_T4_F0[d]	3 ± 5 (0.2)	21 ± 20 (0.5)	43 ± 55 (1.3)	47 ± 23 (1.7)	100 ± 0 (1.9)	32 ± 18 (1.0)	28 ± 12 (0.4)
12M_03_T4_F0[d]	-1 ± 4 (-0.1)	38 ± 7 (0.8)	42 ± 31 (1.3)	15 ± 35 (0.5)	83 ± 29 (1.6)	26 ± 5 (0.8)	71 ± 4 (1.1)
12M_04_T4_F3[d]	8 ± 1 (0.5)	40 ± 14 (0.9)	57 ± 12 (1.7)	39 ± 41 (1.4)	50 ± 50 (0.9)	20 ± 21 (0.6)	72 ± 3 (1.1)
12M_05_T8_F3[d]	19 ± 3 (1.2)	57 ± 2 (1.3)	29 ± 7 (0.9)	37 ± 76 (1.3)	100 ± 0 (1.9)	23 ± 0 (0.7)	82 ± 3 (1.3)
12M_06_T8_F0[d]	27 ± 13 (1.7)	53 ± 7 (1.2)	30 ± 45 (0.9)	32 ± 48 (1.1)	19 ± 24 (0.4)	34 ± 20 (1.1)	74 ± 10 (1.2)
12M_07_T4_F0[d]	25 ± 11 (1.6)	60 ± 13 (1.3)	-2 ± 74 (-0.1)	-25 ± 38 (-0.9)	3 ± 76 (0.1)	29 ± 22 (0.9)	75 ± 14 (1.2)
12M_08_T4_F5[d]	25 ± 14 (1.6)	51 ± 6 (1.1)	51 ± 34 (1.5)	58 ± 57 (2.1)	57 ± 39 (1.1)	38 ± 12 (1.2)	73 ± 11 (1.1)
12M_all data[e]	16 ±15 (1.0)	45 ± 19 (1.0)	33 ± 51 (1.0)	28 ± 48 (1.0)	53 ± 56 (1.0)	31 ± 17 (1.0)	64 ± 22 (1.0)

[a] Results of a MaB-floc sequencing batch reactor of 4 L with hydraulic retention time of 2 days (Van Den Hende et al., submitted a); [b] No data; [c] Values between brackets are scale-up conversion factors calculated as average removal efficiency for a certain reactor divided by the average removal efficiency of 12M_all; [d] Names of reactor operation periods are composed as ‘reactor volume in m^3’M_’operation period’_T‘HRT in days’_F’flue gas flow rate in L min^{-1}’; [e] Average of all samples of period 2-8 of reactor 12M

Table 3. Biomass productivities and quantum yield of MaB-floc SBRs treating aquaculture wastewater and scale-up conversion factors

Reactor	Reactor performances					Scale-up conversion factors compared to 12M_all[b]				
	Volumetric biomass productivity (mg $L_{reactor}^{-1}$ day^{-1})		Production per $V_{influent}$ treated (mg $L_{influent}^{-1}$)		Max. Quantum Yield[a] (%)	Volumetric biomass productivity ratio		Production per $V_{influent}$ treated ratio		Max. Quantum Yield ratio
	TSS	VSS	TSS	VSS		TSS	VSS	TSS	VSS	
4L_T2[c]	236 ± 73	109 ± 30	472 ± 146	218 ± 60	6.5 ± 5.9	10.4	13.4	4.9	6.3	5.9
40L_T2	65 ± 8	45 ± 6	131 ± 16	91 ± 12	11.1 ± 10.1	2.9	5.5	1.4	2.6	10.1
400L_T4_0.75	14 ± 15	11 ± 12	55 ± 60	42 ± 49	4.0 ± 3.6	0.6	1.3	0.6	1.2	3.6
400L_T4_0.50	16 ± 23	12 ± 17	64 ± 92	48 ± 66	4.6 ± 4.2	0.7	1.5	0.7	1.4	4.2
12M_01_T8_F0[d]	4 ± 6	3 ± 4	30 ± 47	25 ±35	1.3 ± 1.1	0.2	0.4	0.3	0.7	1.1
12M_02_T4_F0[d]	7 ± 11	6 ± 9	29 ± 43	23 ± 36	1.3 ± 1.2	0.3	0.7	0.3	0.7	1.2
12M_03_T4_F0[d]	21 ± 20	10 ± 11	83 ± 80	41 ± 44	1.2 ± 1.1	0.9	1.3	0.9	1.2	1.1
12M_04_T4_F3[d]	34 ± 20	15 ± 8	136 ± 81	60 ± 32	1.4 ± 1.2	1.5	1.8	1.4	1.7	1.2
12M_05_T8_F3[d]	17 ± 49	5 ± 40	134 ± 395	43 ± 318	0.6 ± 0.6	0.7	0.7	1.4	1.2	0.6
12M_06_T8_F0[d]	6 ± 100	1 ± 31	45 ± 796	11 ± 246	0.6 ± 0.6	0.2	0.2	0.5	0.3	0.6
12M_07_T4_F0[d]	47 ± 68	12 ± 19	176 ± 276	47 ± 77	1.2 ± 1.1	2.0	1.5	1.8	1.3	1.1
12M_08_T4_F5[d]	33 ± 47	11± 12	132 ± 189	43 ± 50	1.0 ± 0.9	1.5	1.3	1.4	1.2	0.9
12M_all[e]	23 ± 54	8 ± 18	96 ± 337	35 ± 115	1.1 ± 1.0	1.0	1.0	1.0	1.0	1.0

[a] Highest quantum yield that is possible, meaning in case of no heterotrophic growth, no TSS or VSS addition by influent and no MaB-floc grazers; calculated as mol C produced per day on mol photons provided per day, based on 50 % C content of VSS; [b] Scale-up conversion factor as average value for a certain reactor divided by the average value of 12M_all; [c] Results of previous study (Van Den Hende et al., submitted a); [d] Names of reactor operation periods are composed as 'reactor volume in m^3'M_'operation period'_T'HRT in days'_F'flue gas flow rate in L min^{-1}'; [e] Average of all samples of period 1-8 of outdoor reactor 12M

Table 4. Biomass loss during harvesting by settling, gravity filtering and press filtering of outdoor MaB-flocs, and TS and VS content of the resulting dewatered MaB-floc cake

Reactor operation (amount of harvests)	MaB-floc loss (%)								Solid content of MaB-floc cake	
	Settling		Gravity filtering		Press filtering		Total			
	TSS	VSS	TSS	VSS	TSS	VSS	TSS	VSS	(%TS)	(%VS)
12M_04_T4_F3 (1) [a]	4.7	6.3	1.1	1.0	0.00	0.00	5.8	7.4	24.6	no data
12M_05_T8_F3 (2) [a]	13.9 ± 0.3	14.2 ± 8.8	1.9 ± 0.4	2.2 ± 1.0	0.08 ± 0.02	0.08± 0.03	15.9 ± 0.6	16.5 ± 9.9	34.2 ± 2.8	24.8 ± 0.9
12M_06_T8_F0 (3) [a]	15.6 ± 3.2	20.8 ± 4.1	2.7 ± 0.8	3.1 ± 0.9	0.07 ± 0.01	0.12 ± 0.02	18.1 ± 2.4	24.0 ± 3.2	33.7 ± 6.8	23.6 ± 3.0
12M_07_T4_F0 (6) [a]	8.8 ± 4.0	20.2 ± 11.3	1.4 ± 0.7	2.8 ± 1.7	0.05 ± 0.03	0.12 ± 0.06	10.2 ± 4.2	23.1 ± 12.1	49.7 ± 5.5	25.6 ± 3.8
12M_08_T4_F5 (8) [a]	3.3 ± 3.4	6.9 ± 6.1	0.4 ± 0.3	0.9 ± 0.8	0.04 ± 0.04	0.10 ± 0.08	3.7 ± 3.7	7.8 ± 6.8	46.6 ± 3.1	25.7 ±4.8
12M_All (20) [b]	7.9 ± 5.7	13.6 ± 9.8	1.2 ± 0.9	1.9 ± 1.4	0.05 ± 0.03	0.10 ± 0.07	9.1 ± 6.4	15.7 ± 10.9	42.9 ± 8.7	25.3 ± 3.8

[a] Names of reactor operation periods are composed as 'reactor volume in m^3'M_'operation period'_T'HRT in days'_F'flue gas flow rate in L min^{-1}'; [b] Average of all samples of period 1-8 of outdoor reactor 12M

Fig. 1. Set-up of lab-scale (a, b) and outdoor (c) MaB-floc SBRs treating aquaculture wastewater

Fig. 2. Turbidity, COD, BOD and TOC of influent (♦) and effluent (▲) of aquaculture wastewater treating MaB-floc SBRs of 40 L (a), 400 L (b) and 12 m^3 (c)

Discharge norms (- - - - -) according to current (2013/09) environmental permit for this wastewater

Fig. 3. TIC, EC and pH of influent (♦) and effluent (▲) of aquaculture wastewater treating MaB-floc SBRs of 40 L (a), 400 L (b) and 12 m^3 (c)

Discharge norms (- - - - -) according to current (2013/09) environmental permit for this wastewater.

Fig. 4. N and P in influent (♦) and effluent (▲) of aquaculture wastewater treating MaB-floc SBRs of 40 L (a), 400 L (b) and 12 m^3 (c)

Discharge norms (- - - - -) according to current (2013/09) environmental permit for this wastewater.

Fig. 5. Ambient and reactor T (a), reactor DO (b), reactor pH (c) and PPFD (d) of the 12 m^3 outdoor MaB-floc raceway pond

Fig. 6. MaB-floc characteristics of SBRs of 4 L, 40 L, 400 L and 12 m^3: dSVI (a), density of settled MaB-flocs (b), VSS/TSS (c), chlorophyll*a* content (d), density before harvesting (e), density after harvesting (f) and correlation between VSS/TSS and dSVI (g)

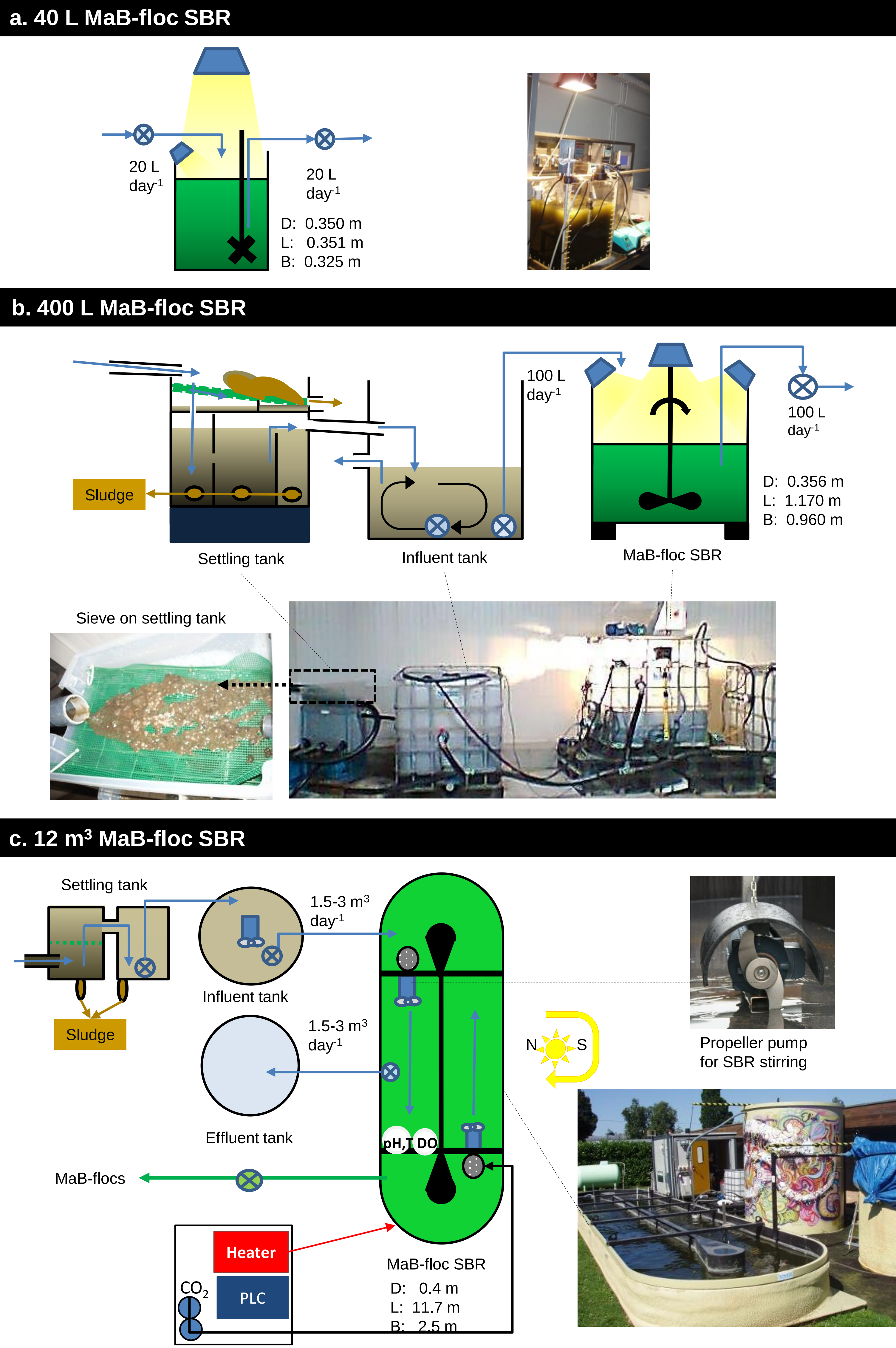
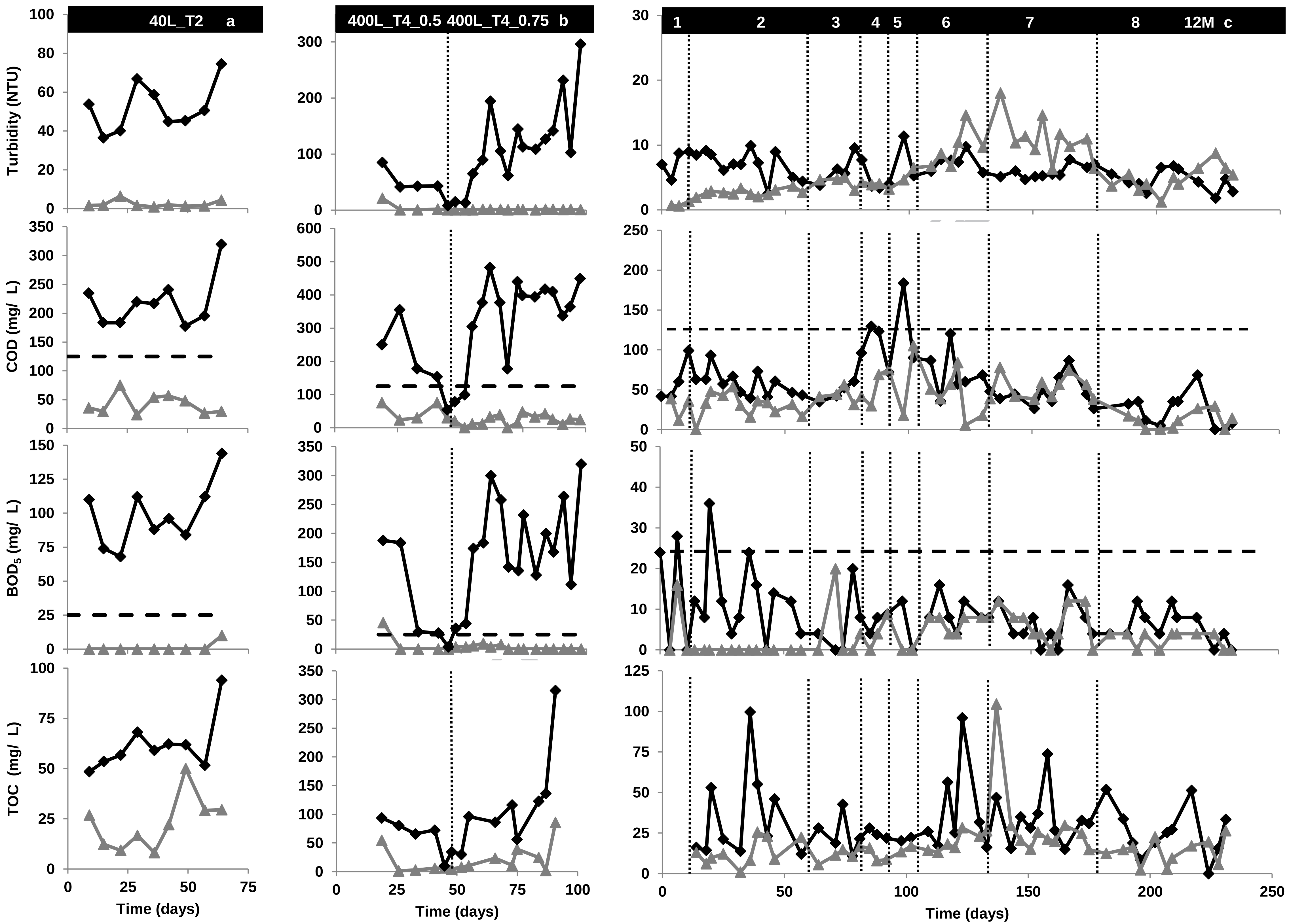

a. 40 L MaB-floc SBR
20 L day-1
20 L day-1
D: 0.350 m
L: 0.351 m
B: 0.325 m
b. 400 L MaB-floc SBR
100 L day-1
100 L day-1
Sludge
D: 0.356 m
L: 1.170 m
B: 0.960 m
Settling tank
Influent tank
MaB-floc SBR
Sieve on settling tank
c. 12 m3 MaB-floc SBR
Settling tank
1.5-3 m3 day-1
Influent tank
Sludge
1.5-3 m3 day-1
N S
Propeller pump for SBR stirring
Effluent tank
pH,T DO
MaB-flocs
Heater
CO2
PLC
MaB-floc SBR
D: 0.4 m
L: 11.7 m
B: 2.5 m

Figure 2 as is pdf



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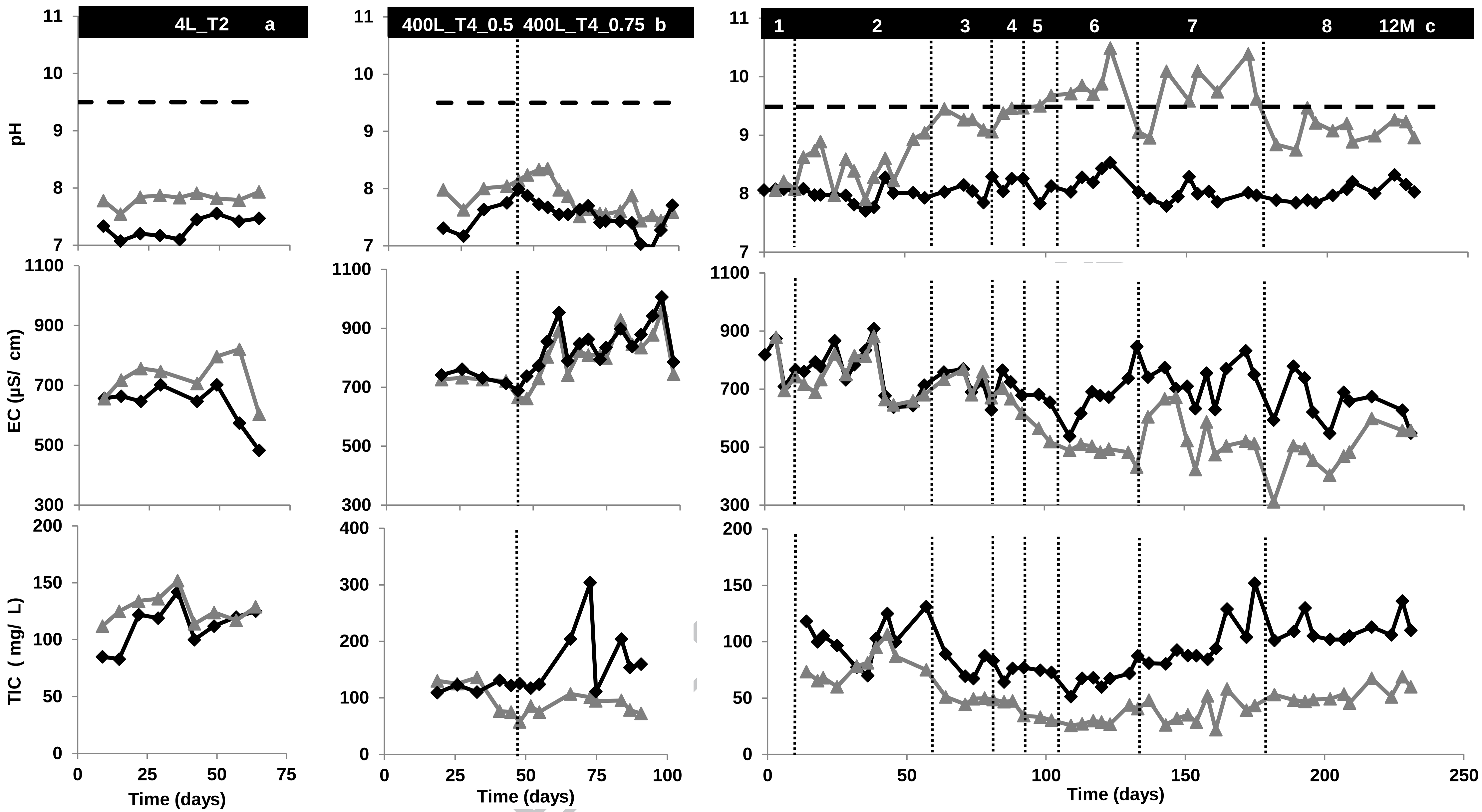

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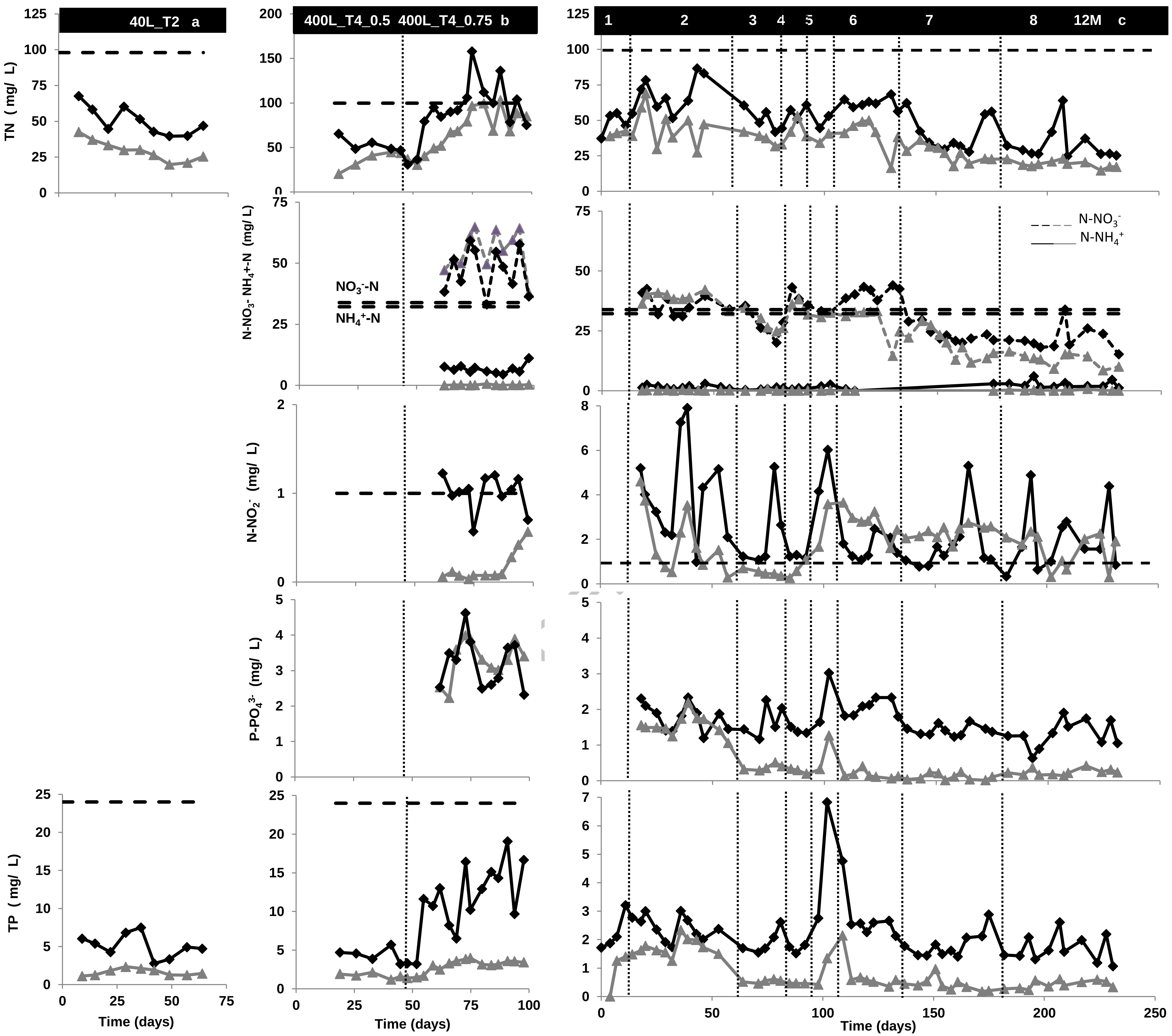

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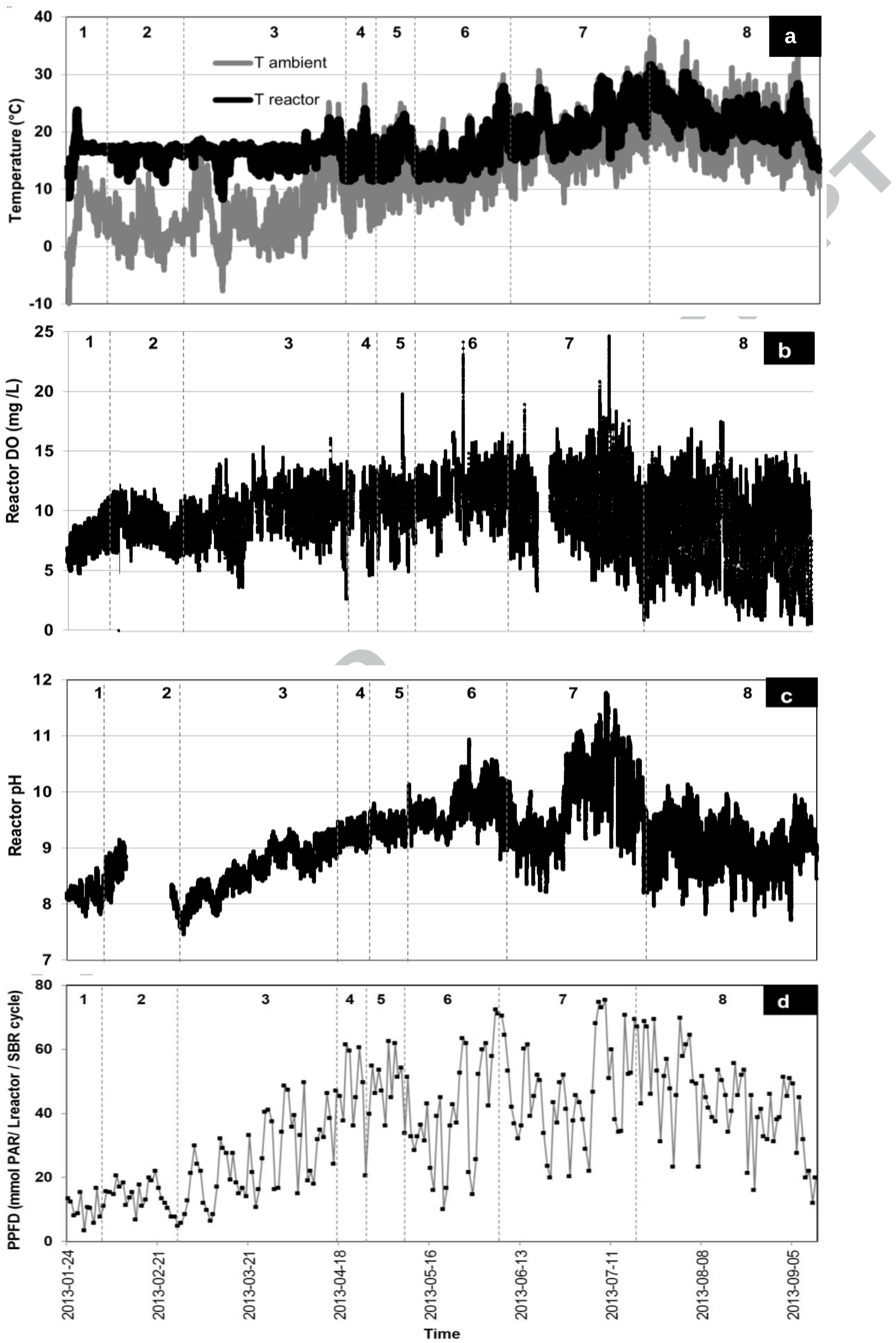

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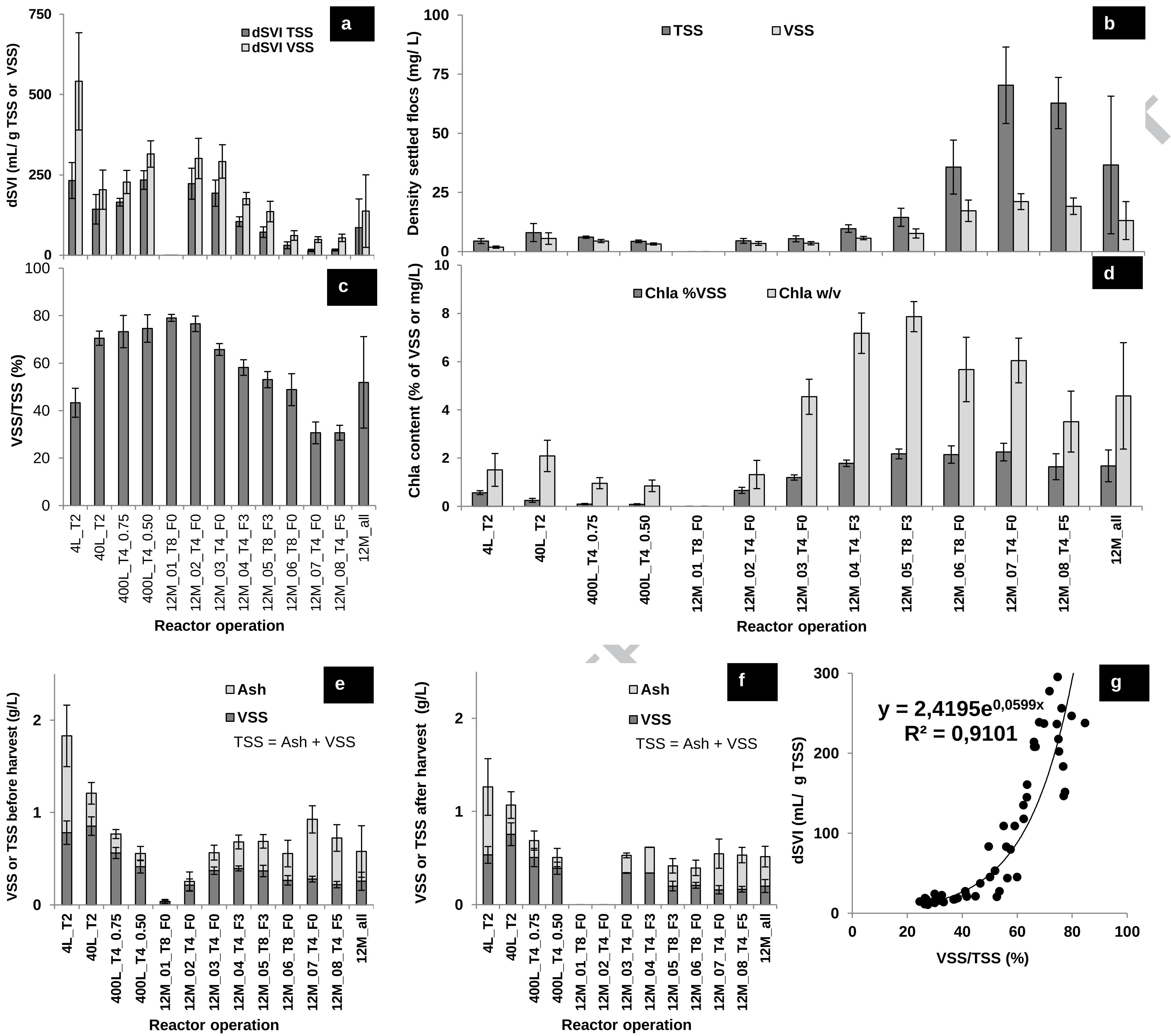

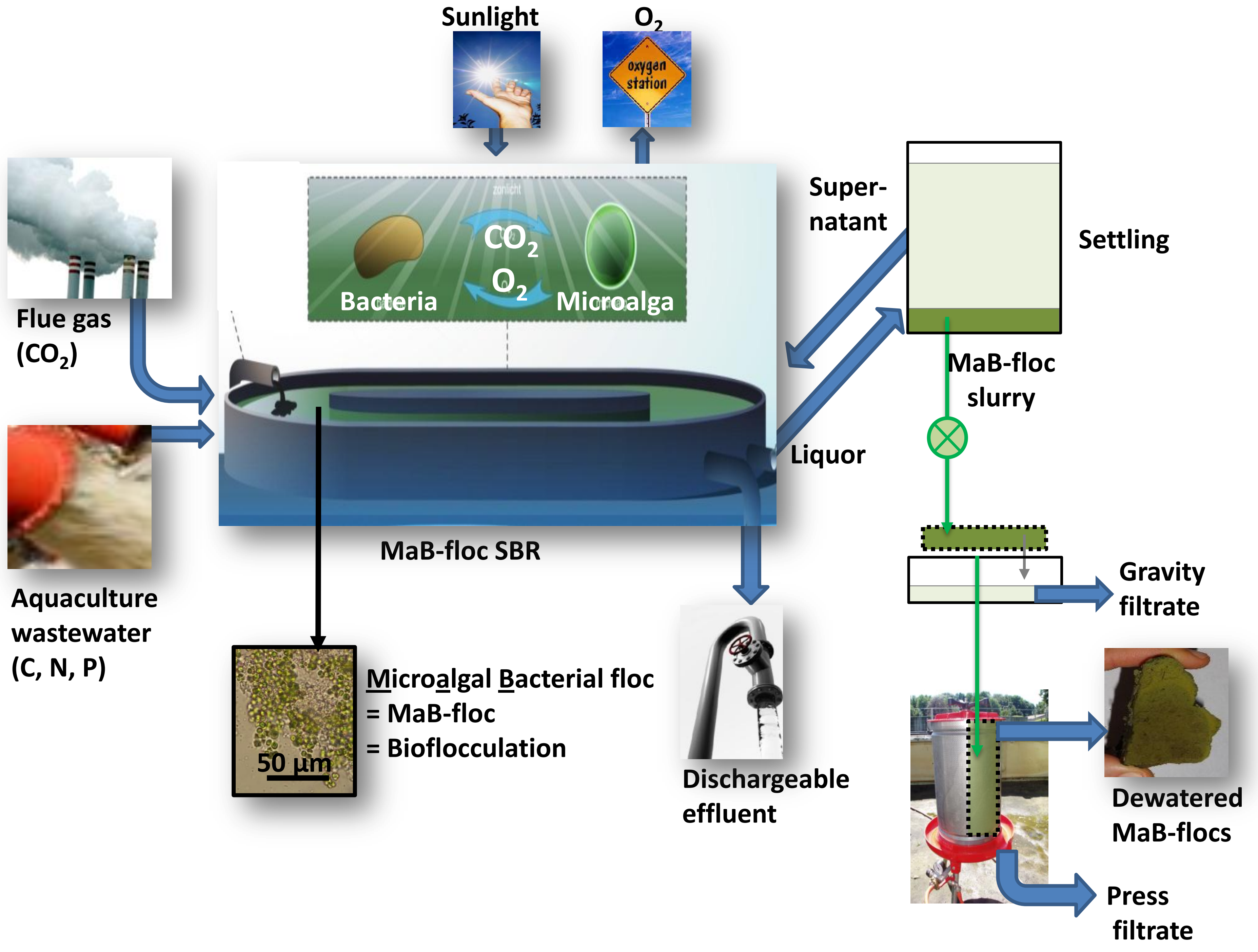

Sunlight
O_2
oxygen station
Flue gas
(CO_2)
Bacteria
CO_2
O_2
Microalga
Super-
natant
Settling
MaB-floc
slurry
Liquor
Aquaculture
wastewater
(C, N, P)
MaB-floc SBR
Gravity
filtrate
50 µm
Microalgal Bacterial floc
= MaB-floc
= Bioflocculation
Dischargeable
effluent
Dewatered
MaB-flocs
Press
filtrate

Highlights

- MaB-floc SBRs were up-scaled from indoor reactors to outdoor raceway pond
- Up-scaling lowered nutrient removal and biomass productivity
- Outdoors, flue gas sparging was needed to obtain a dischargeable effluent pH
- Outdoor operation improved MaB-floc settling and ash content
- MaB-flocs were dewatered by filtering with 99 % recovery to 43 %TS